the extremities, emaciation or hair loss.

Summary. 1. In a group of 7 dogs, the common carotid and vertebral arteries were doubly ligated and sectioned. In a second group of 7 dogs, common carotids were cut in 2 animals, vertebral arteries in another 2, vertebrals and internal carotids in another 2, and common carotids and one vertebral in one dog. 2. Animals in both groups survived 4 months from August to December, 1959. Heart rates, body temperatures and eye-grounds remained essentially normal in animals of both groups. Of dogs in the first group (carotid and vertebral arteries cut), intelligence and alertness appear somewhat impaired, and physical condition is poor, with marked emaciation, hair loss, and edema of the extremities. All animals in second group appear normal in all respects. 3. The presence of known arterial anastomoses makes complete cerebral anemia most unlikely following section of both common carotid and both vertebral arteries in the dog. It is concluded that permanent interruption of the carotid and vertebral arterial supply to the head and brain of the dog is not incompatible with life, and that collateral circulation to the head is adequate to sustain life for at least 4 months.

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Received December 7, 1959. P.S.E.B.M., 1960, v103.

Effect of Insulin on Rate of Hepatic Uptake of NEFA.* (25604)

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Considerable attention has been given to the role of plasma albumin bound free fatty acids (NEFA/UFA) in mobilization and transport of lipids(1). The interrelation of plasma NEFA and carbohydrate has been emphasized(2,3). The observed fall in plasma NEFA in response to insulin has been attributed to an action of insulin to inhibit NEFA release from adipose tissue. This conclusion is based on the evidence: 1) that insulin inhibits release of NEFA from adipose tissue *in vitro*(4); 2) that insulin effects changes in plasma C^{14} NEFA specific activity(5); and 3) that glucose and insulin abolish A-V differences in plasma NEFA(6). Stein and Shapiro injected C^{14} palmitic and linoleic acid into the mesenteric vein of anesthetized rats (7). These studies have demonstrated that the liver rapidly removes labeled fatty acids from plasma and incorporates them into hepatic triglycerides and phospholipids. The present report describes the effect of insulin on the measured rate of removal of plasma NEFA by the liver in normal unanesthetized dogs.

Methods and materials. Eight experiments were performed on 4 male and 4 female mongrel dogs weighing 16 to 22 (average 19) kg. The hepatic vessels were catheterized with large plastic tubing by a previously described technic 2 to 7 days prior to experiments(8). All dogs were studied in an unanesthetized state without sedation after having been fasted overnight. Arterial, portal and hepatic venous blood was withdrawn by a constant aspiration pump, so that 4-6 contemporane-

* Supported by research grants from USPHS and Eli Lilly and Co., Indianapolis, Ind.

ous, 10 minute integrated samples were obtained consecutively before and after insulin administration. In all instances blood from a donor dog was used to replace quantitatively the sampled blood. HGF-free insulin was constantly infused into the portal vein in doses of 0.0016 to 0.0033 u/kg/min. Hepatic plasma flow (HPF) was measured by a modified bromsulphalein method(9). NEFA determinations were made on 1 ml aliquots of the same plasma used for measuring plasma flow. The method of Dole was followed except that the Thymol blue indicator was titrated to the desired end point both before and after addition of the heptane extract. Thus, each sample had, in effect, its own blank. This was done in order to increase the accuracy of the concentration gradients measured on relatively small samples. Data presented here include: 1) measured concentrations of NEFA in arterial, portal and hepatic venous plasma; 2) portal-hepatic venous and arterial portal venous concentration gradients; 3) estimation of rate of hepatic uptake of NEFA obtained by multiplying the portal-hepatic venous plasma NEFA gradient by the HPF; 4) an approximation of output of NEFA from the splanchnic area. By making the assumption that ratio of portal venous blood flow to hepatic arterial blood flow is constant during the experiment, it is possible to obtain a first approximation of rate of splanchnic NEFA output. Blalock and Mason(10) found in the unanesthetized dog that portal vein blood flow comprised an average of 80.5% of total hepatic blood flow. NEFA output therefore was calculated by multiplying the arterial-portal venous plasma NEFA gradient by 80.5% of total hepatic plasma flow.

Results. Mean arterial plasma concentration of NEFA for the series was .84 meq/l during the control period. During the first hour after insulin administration, average arterial plasma NEFA concentration fell to .72 meq/l. The lowest arterial concentration for the series during this period averaged 0.55 meq/l. With one exception this nadir occurred at, or near, the end of the hour. Mean portal and hepatic venous plasma NEFA concentrations are illustrated in Fig. 1.

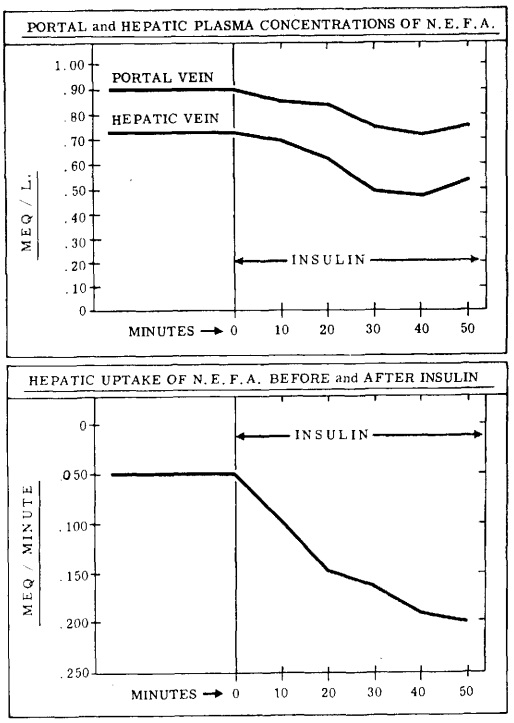

FIG. 1 (top). Mean values of portal and hepatic venous NEFA concentration before and after insulin administration.

FIG. 2 (bottom). Mean rate of hepatic uptake of NEFA during control period and as function of time after insulin administration.

Plasma concentration gradients of NEFA across the liver and across the non-hepatic splanchnic area are summarized in Table I. Mean hepatic portal-venous concentration gradient was negative in the control period (−.016 meq/l), indicating that the liver was taking up NEFA. This negative gradient increased to a mean of −.021 meq/l after insulin administration. Mean portal-arterial concentration gradient was obtained in 6 dogs of the series during the control period; in 3 of these dogs the gradient was positive; in 3, it was negative. In the control period mean portal-arterial gradient was .020 meq/l. After insulin this gradient decreased to a mean of −.002 meq/l.

When the values of all animals were taken together, there were sufficient observations both in the control period and after insulin administration for statistical evaluation. Expressed as mean ± S.E. calculated hepatic uptake of NEFA was 0.049 ± .016 meq/min

TABLE I. Mean Concentrations, Gradients and Output of NEFA before and after Insulin.

	Control	After insulin	Net change
NEFA portal vein	.90 meq/l	.77 meq/l	−.13 meq/l
" hepatic "	.74	.56	−.18
" arterial "	.84	.72	−.12
Hepatic-portal	−.16	−.22	−.06
Portal-arterial*	+.03	−.01	−.04
Hepatic NEFA output†	−.049 ±.016 meq/min.‡	−.150 ±.025 meq/min.‡	−.101 meq/min.‡
Splanchnic NEFA output†	+.020 "	−.002 "	−.022 "

* Based on 6 experiments only.

† Means and S.E. derived by considering all control observations of series together and comparing these with all observations made in experimental period.

‡ Uptake is represented as a negative output or gradient.

before and 0.150 ± .025 meq/min after insulin (P .001). Changes in hepatic NEFA uptake as a function of time after insulin are illustrated in Fig. 2.

The mean NEFA output of the non-hepatic splanchnic area was +.020 ± .016 meq/min before and −.002 ± .017 meq/min after insulin, an increment of +.022 meq/min over control levels. This change was not statistically significant.

Discussion. Studies with labeled fatty acids have indicated that these substances are rapidly removed from the blood and converted in part to triglyceride, presumably in the liver (7,11). The studies presented here have attempted to quantify rate of removal of NEFA by the liver in a control period before and after insulin administration. They indicate that after an infusion of glucagon-free insulin there is an immediate and pronounced increase in rate of hepatic removal of NEFA from portal blood. If it may be assumed that arterial NEFA concentration represents that of the extracellular phase and that the latter comprises 30% of total body weight, calculation of mean decrease of total extracellular NEFA is permitted. The calculated decrease in this series was found to be 0.68 meq. The mean net increase in hepatic NEFA uptake over that of the control period in this series was found to be 0.61 meq after insulin administration. Thus, the increase in rate of NEFA uptake by the liver was almost enough (90%) to account for the decrease in total plasma and extracellular NEFA observed in this series during the first hour after insulin administration.

In the data presented herein, the authors have been unable to find a statistically significant change in output of NEFA from the non-hepatic splanchnic area after insulin administration. These conclusions differ with those of other workers who have concluded from turnover studies and *in vitro* systems that the fall in plasma NEFA is due to a diminution of NEFA released from adipose tissue(4,5). The non-hepatic splanchnic area is comprised of intestinal tract, parenchymal organs as pancreas and spleen, and large stores of mesenteric and omental fat. The gradient, as well as net output or uptake of this area, therefore, represent the influence of both the gut and the intraperitoneal fat stores. The assumption has frequently been made that this splanchnic area is metabolically inactive in the fasting state. However, it has been demonstrated that this area is remarkably active in taking up glucose after insulin administration(12). We feel that at present time there are insufficient data to measure concomitantly the relative contribution of liver and body adipose tissue, *per se,* to the fall in plasma NEFA after insulin in the *in vivo* state.

An immediate effect of insulin which modifies hepatic lipid metabolism has been noted in a number of instances (13,14,15). If there is an action of insulin which increases hepatic fatty acid synthesis, such an effect would not necessarily be expected to increase NEFA uptake by the liver. If, however, fatty acid synthesis is stimulated by an increase in rate of esterification to triglyceride, as suggested by Balmain, *et al.*(16,17) for mammary tissue, then it is reasonable to expect that an increase in triglyceride formation would increase hepatic NEFA clearance. On the basis of these data one may be led to speculate or predict that there may be an increased hepatic output of triglycerides concomitant with the in-

creased uptake of NEFA following administration of insulin.

Summary. Concentrations of NEFA in plasma of blood obtained from splenic artery, portal and hepatic veins were measured in unanesthetized dogs together with hepatic plasma flow. Rates of hepatic and non-hepatic splanchnic uptake or output of NEFA were calculated before and after insulin administration. A marked increase in rate of hepatic uptake of NEFA was observed after insulin administration. No significant change in rate of NEFA output from non-hepatic splanchnic area was found.

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Received December 8, 1959. P.S.E.B.M., 1960, v103.

Effects of Physico-Chemical State of Vit. B_{12} Preparation in Digestive Tract on Its Absorption.* (25605)

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D-sorbitol in large doses can enhance intestinal absorption of Vit. B_{12} under appropriate conditions in man(1) and rats(2). This phenomenon may be (a) due to formation of a chemical derivative of sorbitol in the gastrointestinal tract which can enhance absorption (3), or (b) due to physical characteristics of the concentrated sorbitol solution. Therefore, some commercially available surfactants which are derivatives of sorbitol and the importance of the physical characteristics of the administered solution in enhancement of Vit. B_{12} absorption were studied. This communication reports the results of such studies and presents a hypothesis on the mechanism of enhancement of Vit. B_{12} absorption by such compounds.

Methods. Measurement of Vit. B_{12} absorption. Adult rats were fed by stomach tube 50 mμg or 1000 mμg of aqueous radioactive $Co^{60}B_{12}$ with a total radioactivity of 50 and 100 mμc, respectively. Absorption was measured by fecal recovery of radioactivity in 4 days, or only by organ uptake of radioactivity 2 days after administration when the latter dose was used. Determination of radioactivity in feces and organs has already been described(4). In some instances "Intestinal Loop" technic(5) was used.

Results. Effect of surface active agents on Vit. B_{12} absorption.† One group of rats was given by a stomach tube 1 cc of undiluted Tween 80 and immediately afterwards 1 cc of $Co^{60}B_{12}$ by another stomach tube. Another group of control animals received

* Supported by Grant-in-Aid from U.S.P.H.S.

† Surface active agents used were obtained from Atlas Powder Co.